ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

SELECTIVE INHIBITION PATTERNS OF SUCCINIC DEHYDROGENASE AND LOCAL NECROBIOSIS IN TUBULES OF RAT KIDNEY INDUCED BY SIX MERCURIAL DIURETICS

BY

KIMMO K. MUSTAKALLIO and ANTTI TELKKÄ

VOL. 33 1955 SUPPLEMENTUM 1

MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

SELECTIVE INHIBITION PATTERNS OF SUCCINIC DEHYDROGENASE AND LOCAL NECROBIOSIS IN TUBULES OF RAT KIDNEY INDUCED BY SIX MERCURIAL DIURETICS

With 9 Plates

BY

KIMMO K. MUSTAKALLIO and ANTTI TELKKÄ

HELSINKI 1955

HELSINKI 1955
MERCATORIN KIRJAPAINO

SELECTIVE INHIBITION PATTERNS OF SUCCINIC DEHYDROGENASE AND LOCAL NECROBIOSIS IN TUBULES OF RAT KIDNEY INDUCED BY SIX MERCURIAL DIURETICS

by

KIMMO K. MUSTAKALLIO and ANTTI TELKKÄ

(Received for publication December 22, 1954)

In previous publications (9, 10, 22), we have reported that the administration of a mercurial diuretic, mercurophylline (Novurit), was followed by a histochemically demonstrable reduction in the succinic dehydrogenase activity of rat kidney tubules. The thick ascending limbs of Henle's loops and apparently the straight terminal portions of the proximal convoluted tubules were the sites of the most pronounced inhibition by this mercurial. The activity of the proximal and distal convoluted tubules was less involved.

Later, two groups of investigators (16, 25, 26) restudied this question employing another mercurial diuretic, meralluride sodium (Mercuhydrin Sodium). Meralluride caused a marked depression of succinic dehydrogenase in the proximal convoluted tubules, while other portions of the nephron were little affected. The thick ascending portions of Henle's loops were reported to retain their full dehydrogenase activity (25).

The highly divergent results obtained with these two mercurials prompted us to compare the inhibitory properties of six different mercurial diuretics and in addition that of mercuric chloride. This led to the discovery of the selective inhibition patterns of succinic dehydrogenase and of the local necrobiotic changes induced by these mercurials.

EXPERIMENTAL

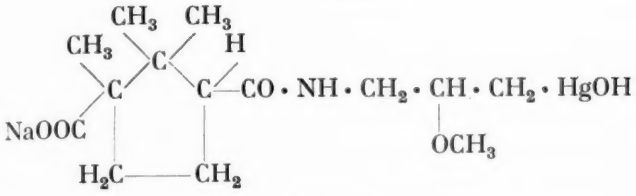
The series examined consisted of 140 male albino rats of the Wistar strain, weighing 100 to 125 g. Ten rats and an equal number of controls were used for testing the effects of each of the seven mercurials.

The mercurial diuretics used were Novurit »Medica», the brand of mercurophylline (control No. 5402), Thiomerin Sodium »Wyeth», the brand of mercaptomerin (control No. 1532388), Diurgan »Bayer Products Ltd.», the brand of mersalyl (control No. G.C. 420), Esidron »Ciba» (control No. 47657), Mercuhydrin Sodium »Lakeside Laboratories Inc.», the brand of meralluride (control No. 2371) and Neohydrin »Lakeside Laboratories Inc.» (control No. 2401). Novurit, Diurgan, Esidron, and Mercuhydrin Sodium contained also a molecular equivalent of theophylline.

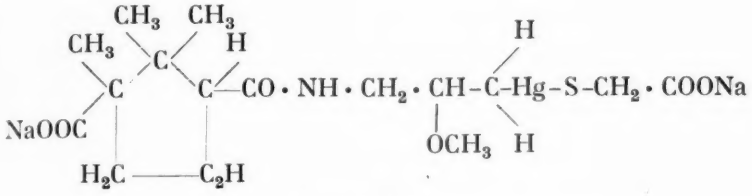
The structural formulas of the six mercurial diuretics are presented in Table I. All the drugs were used on the basis of a mercury content of approximately 40 mg/cc. The mercurials were administered subcutaneously with a microsyringe. The doses used corresponded to 7, 15, 30 and 45 mg of mercury per kilogram of body weight. In doses of 30 and 45 mgHg/kg, sublimate and mersalyl were found to be generally lethal within 24 hours, and therefore, their dosage was reduced to correspond to 5, 10 and 15 mgHg/kg. The rats were fasted throughout the experimental period, but water was provided *ad libitum*. After 4, 6 or 24 hours the animals were killed by decapitation without any anaesthesia. A piece from the immediately removed kidney was sectioned simultaneously with a corresponding control specimen with a freezing-microtome at 40 μ. The frozen sections were dipped directly from the cooled knife into their respective incubation vials kept at a constant temperature of 38°C. The incubation mixture was prepared according to Seligman and Rutenburg (19). Neotetrazolium (NT) manufactured by Pannone Chemicals Co., Verona, N. J., and blue tetrazolium (BT) from Dajac Laboratories, Monomer-Polymer Inc., Leominster, Mass., were used.

In order to demonstrate pathological changes, kidney pieces were fixed in 10 per cent formalin, embedded in paraffin, sectioned, and stained with hemalum-eosin. Also histochemically demonstrable sulfhydryl groups were studied in formalin fixed paraffin

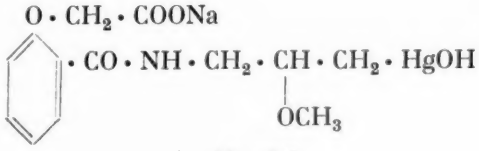
TABLE I



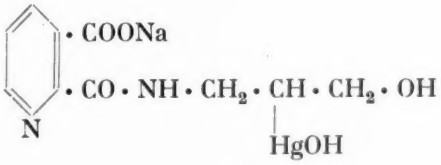
Mercurophylline



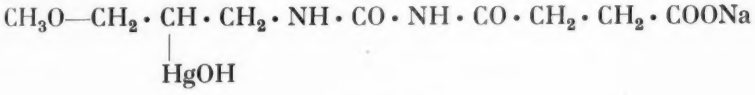
Mercaptomerin



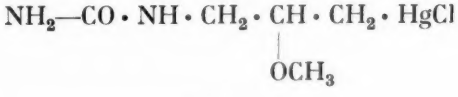
Mersalyl



Esidron



Meralluride



Neohydrin

sections (13) using Bennett's (1) reagent (Bios Laboratories Inc., New York, N.Y.) as a saturated solution in 80 per cent ethanol.

Furthermore, the kidneys of some animals of each group were examined for alterations in the functioning blood vessels. The thioflavin-S-method introduced by Schlegel (18) was applied according to the modification of Oliver and associates (11). Four per cent aqueous solution of Thioflavin-S (G. Grübler & Co., Berlin-West) was injected intravenously under aether anaesthesia in a dose of 1 cc/kg within a period of three seconds. The kidney was removed at a measured time interval of eight seconds from the beginning of the injection, immediately frozen and sectioned at 50 μ. The sections were mounted with pure glycerin on non-fluorescent slides and examined thereafter with a fluorescent microscope.

Attempts were made to demonstrate free mercuric-ions splitted from the compounds by reducing them with stannous chloride to metallic mercury according to the histochemical method proposed by Hand and co-workers (4). In addition, efforts were made to localize the mercuric-ions by complexing them with diphenylthiocarbazone (Merck) in an acetone-water solution according to the method of Mager et al. (8) originally used for the histochemical demonstration of zinc.

NORMAL DISTRIBUTION OF SUCCINIC DEHYDROGENASE ACTIVITY IN TUBULES OF RAT KIDNEY

Great difficulties are encountered in the identification of the different tubular portions from frozen sections stained with the granular deposits of formazan. Although the illustrations of the various investigators reveal an identical distribution pattern of succinic dehydrogenase, there exist divergent opinions as to the nature of the different, more or less active, tubular portions. An initial portion of the proximal convoluted tubule seems to display an intense activity while the straight terminal portion presents only a moderate staining (12). The views begin to differ as regards the activity of the thin descending limb of Henle's loop. According to Rutenburg and co-workers (17) the descending limb of Henle's loop reacts most intensely of all tubular segments, whereas the majority of investigators (3, 14, 16, 26) deny these segments an intense activity. The former view seems to be untenable since the

thin limbs lie to a great part in the unstained papilla of rat kidney. Furthermore, when α-glycerophosphate dehydrogenase (14) and triphosphopyridine nucleotide diaphorase (3) are demonstrated using tetrazolium salts as hydrogen acceptors, the thin limbs are delicately delineated from the surrounded tubules by their high activity throughout their course, a staining pattern entirely different from that seen when succinic dehydrogenase is demonstrated. The thick ascending limb of Henle's loop exhibits a very intense activity according to most investigators (3, 14, 16, 26). Rutenburg et al. (17), however, attribute only a moderate activity to the thick ascending limbs. In any case, an abrupt transition from non-reactive tubular segments to thicker highly active tubules is seen in the outer medullary zone where the thin limbs of Henle are transformed to the thick ascending portions. The view of Padykula (12) that this transitional zone which is sharply demarcated from the unstained papilla represents an acute decrease in the activity of the collecting tubules seems to be untenable. Our earlier expression that the loops of Henle bent to re-enter the kidney cortex at this level is also erroneous as regards the kidney of the rat. The distal convolutions seem to exhibit less of succinic dehydrogenase activity than the initial portions of proximal convolutions (14), but they are also regarded somewhat more active than the proximal convolutions (16). The opinions considering the activity of the initial portions of the collecting tubule differ too. Some of the investigators ascribe a high activity to this segment (12, 14), whereas others regard it as inactive or only feebly active (3, 16, 17, 26).

The following distribution of succinic dehydrogenase activity in the nephron of rat kidney is presented on the basis of an analysis of the pertinent literature and of our own experience gained from the thorough examination of some thousands of kidney sections incubated for succinic dehydrogenase and from their correlation to the routinely stained sections from normal and locally damaged kidneys (Fig. 1—17). In the nearest vicinity of the unstained glomeruli an apparently initial portion of the proximal convoluted tubule exhibited a very intense activity by its dense granulation, whereas the activity in the middle part of this convolution was somewhat less intense. The straight terminal portion of the proximal tubule showed a moderate activity. The thin limb of Henle's loop

was essentially negative while the activity in the thick ascending limb was very intense being highest at the beginning of this segment. The distal convoluted tubule displayed an intense activity similar to that of the middle part of the proximal convolutions and they could hardly be differentiated from each other. The collecting duct was feebly active in the cortex, being negative in the papilla.

Attempts were made to ascertain the correctness of the presented localization of succinic dehydrogenase activity by macerating incubated slices in concentrated hydrochloric acid in order to separate nephrons by microdissection. The formazans of NT and BT, however, dissolved during maceration.

The three segments of the proximal convoluted tubule with different activities of succinic dehydrogenase seem to correspond with the three segments which Suzuki differentiated by their ability to concentrate intravitally injected lithocarmin (21).

THE EFFECTS OF THE MERCURIALS ON DIFFERENT TUBULAR SEGMENTS

The following observations are based on the examination of NT-preparations and on their comparison with the hemalum-eosin sections.

The experimental data are summarized in Table II.

Initial Part of the Proximal Convoluted Tubule. — This part of the proximal tubule was least influenced by the mercurials. In the doses used only meralluride and neohydrin caused, within 6 hours, a clear depression in the very intense succinic dehydrogenase activity of this portion. The corresponding hemalum-eosin sections revealed slightly to moderately damaged cells in this segment. The other mercurials inhibited in a much lesser degree the succinic dehydrogenase activity of this initial part.

Middle Part of the Proximal Convoluted Tubule. — Mercaptomerin, meralluride, neohydrin and mersalyl inhibited strongly the succinic dehydrogenase of this part of the proximal tubule. The highest doses caused a complete lack of formazan staining and necrosis in these tubular portions. Esidron, mercurophylline and sublimate reduced moderately the succinic dehydrogenase activity and caused only slight cellular damage in corresponding doses.

Straight Terminal Portion of the Proximal Convoluted Tubule. — This portion was, in general, most seriously injured by the mer-

Fig. 1. — Succinic dehydrogenase activity in normal rat kidney. 50 ×.

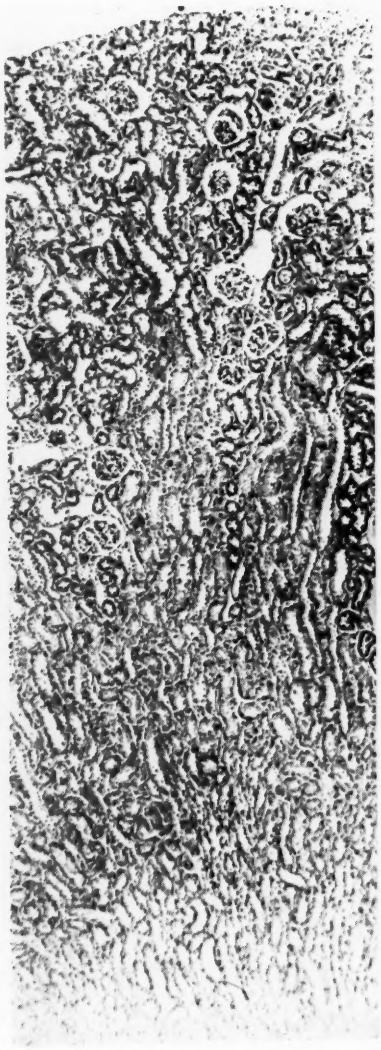


Fig. 2. — Normal rat kidney. Hemalum-eosin. 50 ×.

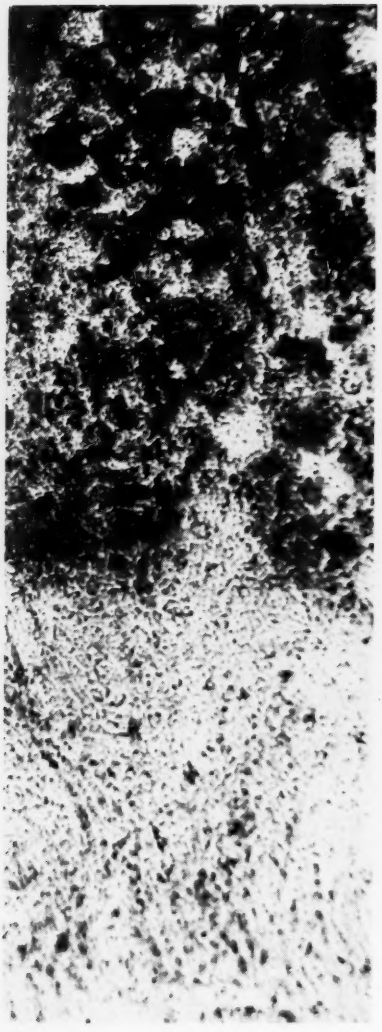


Fig. 3. — Succinic dehydrogenase activity in rat kidney 24 hours after administration of mercurophylline in a dose of 15 mg/Hg/kg. 50 ×.

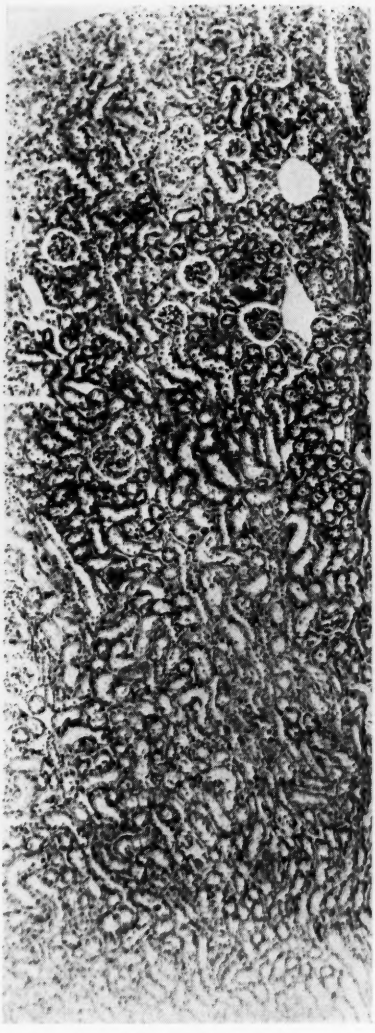


Fig. 4. — The same kidney stained with hemalum-eosin. 50 ×.

Fig. 5. — Succinic dehydrogenase activity in rat kidney 24 hours after administration of mercaptomerin in a dose of 30 mg/Hg/kg. 50 ×.

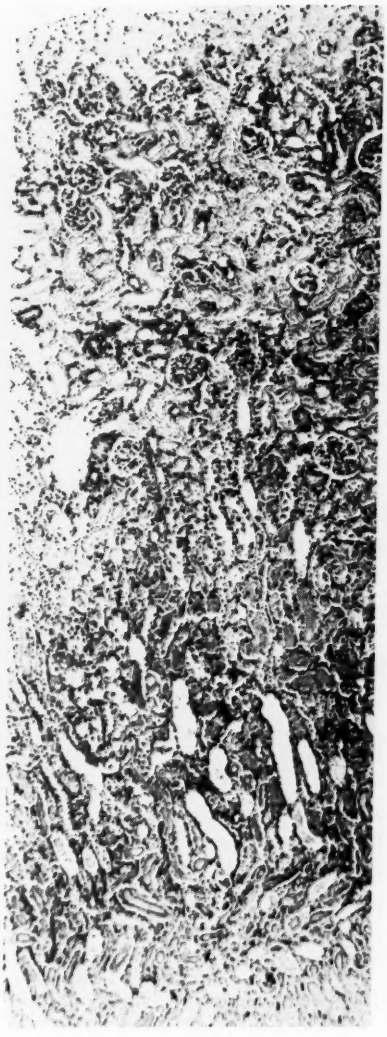


Fig. 6. — The same kidney stained with hemalum-eosin. 50 ×.

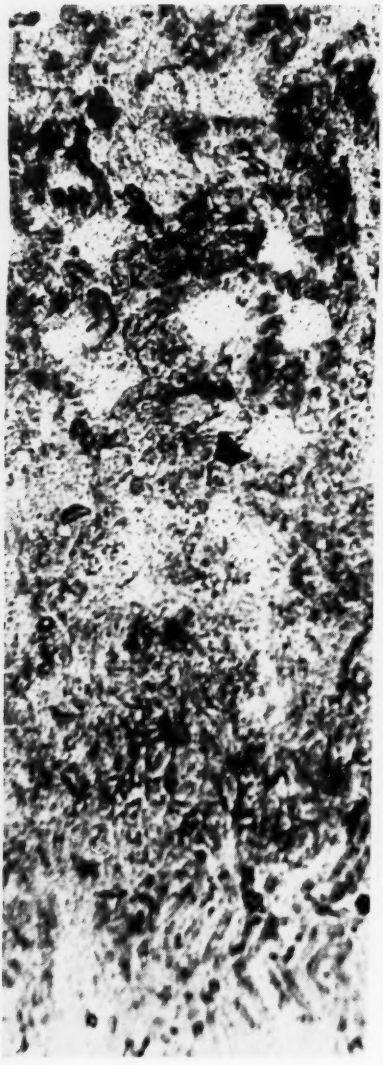


Fig. 7. — Succinic dehydrogenase activity in rat kidney 24 hours after administration of mersalyl in a dose of 15 mg/Hg/kg. 50 ×.

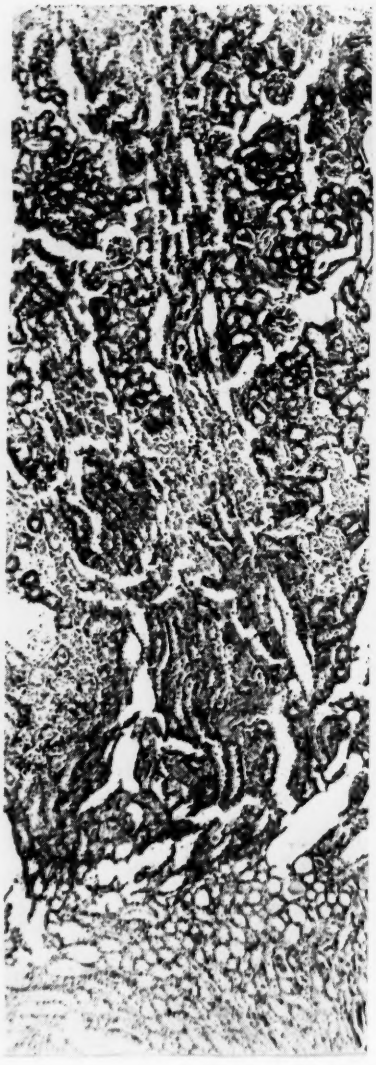


Fig. 8. — The same kidney stained with hemalum-eosin. 50 ×.

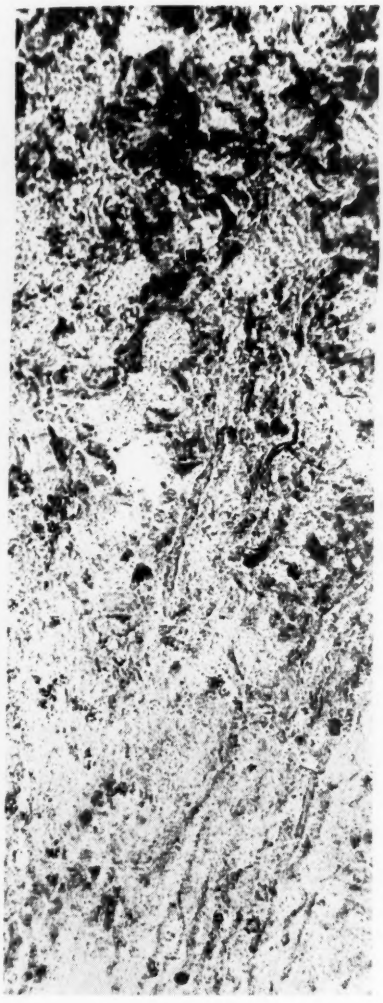


Fig. 9. — Succinic dehydrogenase activity in rat kidney 24 hours after administration of esidron in a dose of 15 mg/Hg/kg. 50 ×.

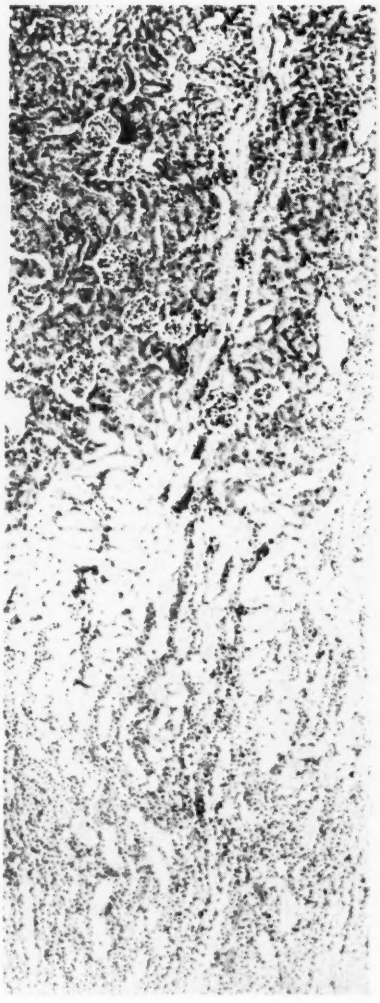


Fig. 10. — The same kidney stained with hemalum-eosin. 50 ×.

Fig. 11. — Succinic dehydrogenase activity in rat kidney 24 hours after administration of meralluride in a dose of 30 mg/Hg/kg. 50 ×.

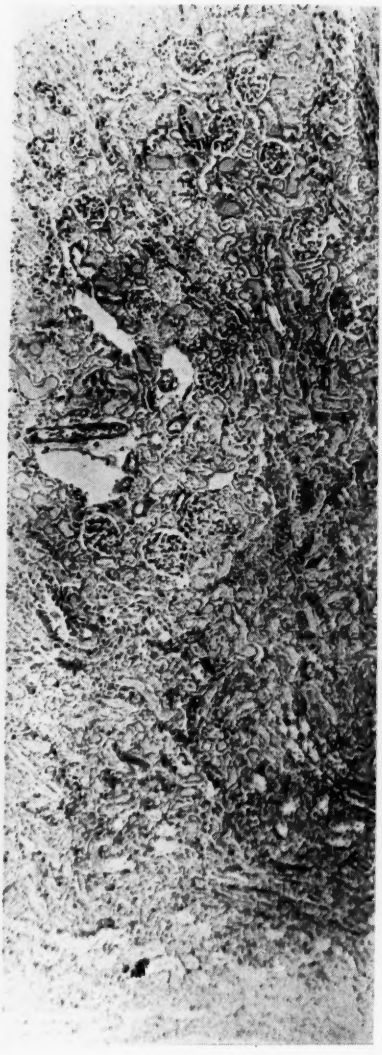


Fig. 12. — The same kidney stained with hemalum-eosin. 50 ×.

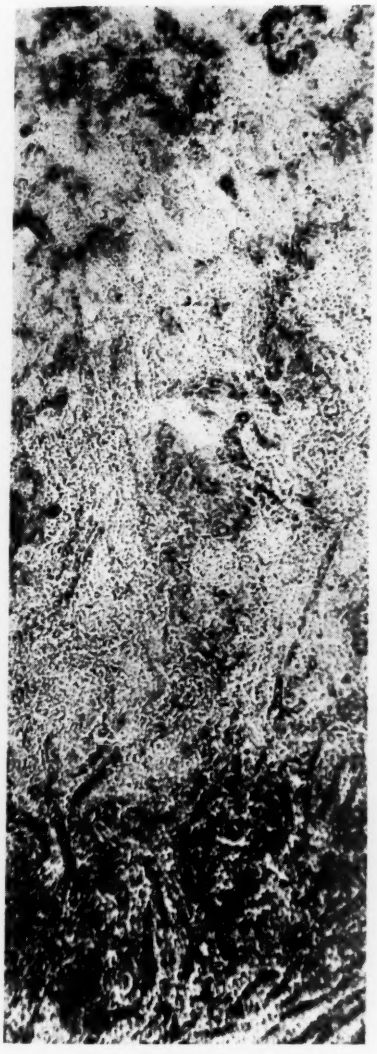


Fig. 13. — Succinic dehydrogenase activity in rat kidney 24 hours after administration of neohydrin in a dose of 15 mg/Hg/kg. 50 ×.

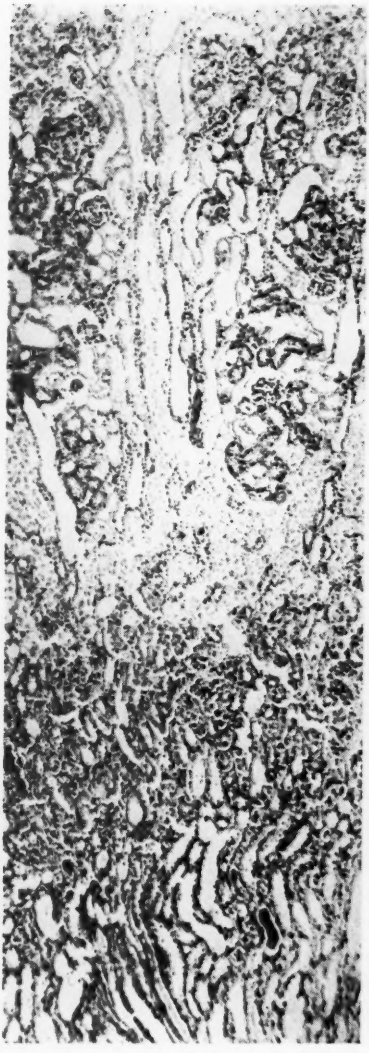


Fig. 14. — The same kidney stained with hemalum-eosin. 50 ×.

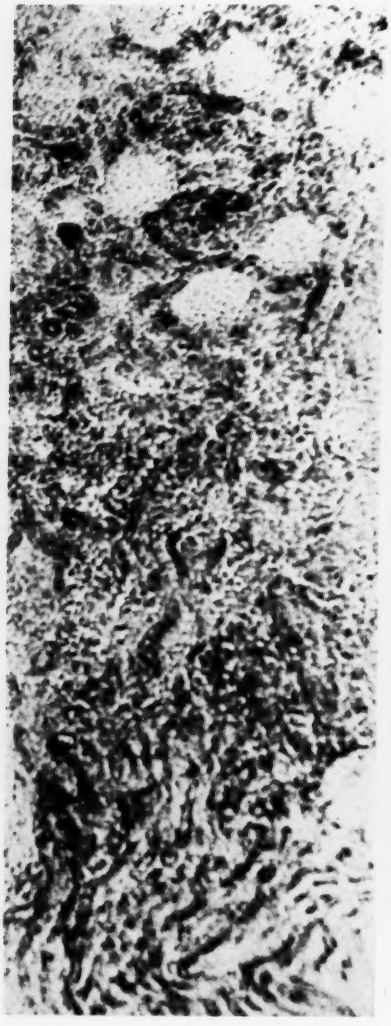


Fig. 15. — Succinic dehydrogenase activity in rat kidney 24 hours after administration of sublimate in a dose of 15 mg/Hg/kg. 50 ×.

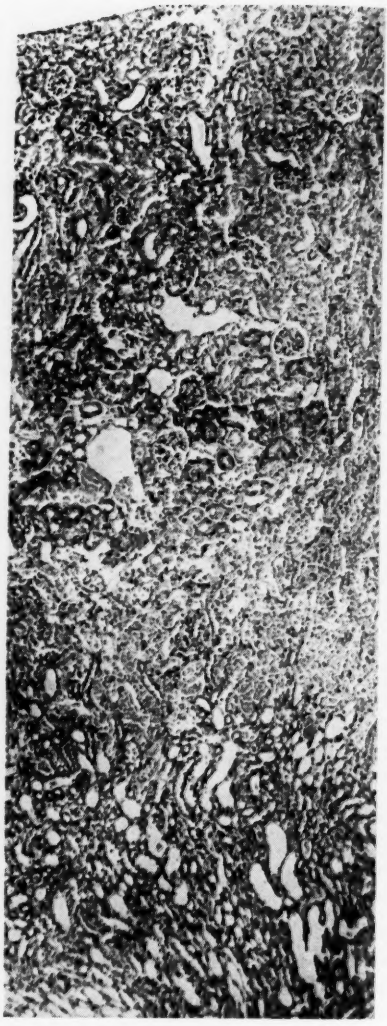


Fig. 16. — The same kidney stained with hemalum-eosin. 50 ×.

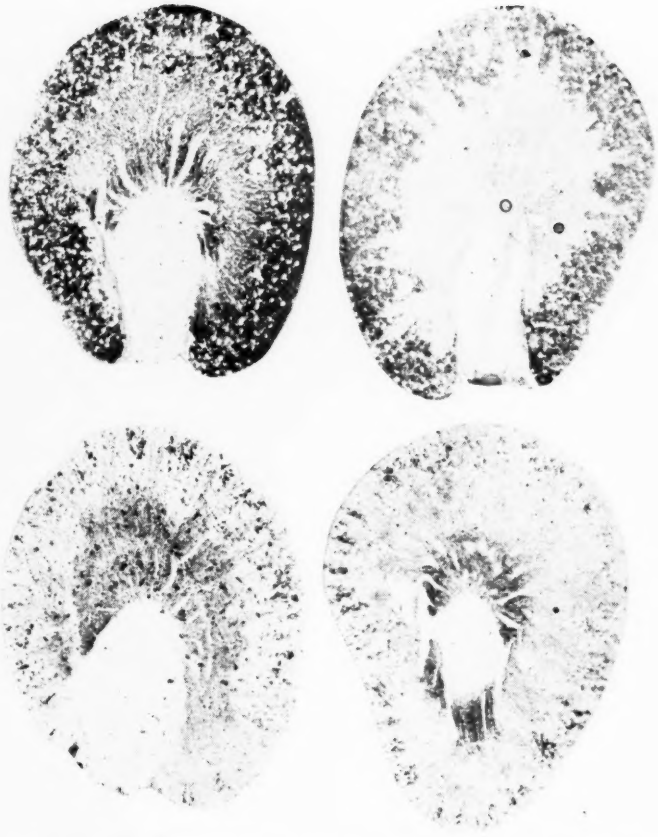


Fig. 17. — Patterns of selective inhibition of succinic dehydrogenase by different mercurial diuretics. At the left, control and mercaptomerin, at the right, mercurophylline and meralluride. The treated animals killed 24 hours after administration of mercurial diuretics in the dose of 30 mg/Hg/kg.

TABLE II

HANGES CAUSED BY MERCURIALS IN SUCCINIC DEHYDROGENASE ACTIVITY AND IN CELLULAR STRUCTURE OF RAT KIDNEY

The range of succinic dehydrogenase activity (SD) is numbered from 0 to 3. The numbers indicate the intensity of activity (staining): 0 no demonstrable, 0—1 weak (variable), 1 moderate, 2 fairly intense, 3 very intense. The changes in cellular structure (CS) are designated as follows: — no discernible changes, ± swelling and occasionally vacuoles, + more extended vacuolization, ++ in addition, pyknotic nuclei, +++ necrosis.

Mercurial	Dose mgHg/kg	Duration in Hours	Proximal Convoluted Tubule						Thick Limb of Henle's Loop		Distal Convoluted Tubule	
			Initial		Middle		Straight Terminal					
			SD	CS	SD	CS	SD	CS	SD	CS	SD	CS
Controls	—	—	3	—	2	—	1	—	3	—	2	—
Mercurophylline	7	24	2	—	1	±	0	++	1	—	1—2	—
	15	4—6	3	—	2	—	0—1	—	1—2	—	2	—
	15	24	2	—	1	±	0	++	0—1	—	1—2	—
	30	4—6	2—3	—	1—2	—	0—1	±	1	—	1—2	—
	30	24	2	±	1	±	0	+++	0	±	1—2	—
	45	4—6	2—3	—	1	—	0	+	0—1	—	1—2	—
	45	24	2	±	1	±	0	+++	0	±	1	±
Mercaptomerin	7	24	2—3	±	0—1	++	1	±	2	—	2	—
	15	4—6	2—3	±	0—1	++	1	±	2—3	—	2	—
	15	24	2	±	0—1	++	1	±	1—2	—	2	—
	30	24	2	±	0	+++	0—1	+	0—1	±	1—2	±
	45	24	1—2	+	0	+++	0—1	+	0—1	±	1—2	±
Mersalyl	5	24	2—3	—	1	+	0 (—1)	++	2	—	2	—
	10	24	2—3	±	0—1	++	0 (—1)	++(+)	2	—	2	—
	15	4—6	2—3	±	0—1	++	0 (—1)	++	2	—	2	—
	15	24	2	±	0—1	++	0	+++	1—2	±	2	—
Esidron	7	24	2—3	—	1	±(+)	0	+++	2	—	2	—
	15	4—6	3	—	1	±	0 (—1)	++(+)	2—3	—	2	—
	15	24	2	±	0—1	±(+)	0	+++	1—2	—	2	—
	30	24	2	±	0—1	+	0	+++	1—2	—	2	—
	45	24	1—2	+	0	++(+)	0	+++	1	±	1—2	±
Meralluride	7	24	0—1	++(+)	0	+++	0	+++	3	—	1—2	±
	15	4—6	2	+(+)	0—1	++	0—1	++(+)	3	—	2	—
	15	24	0—1	++(+)	0	+++	0	+++	1—3	±	1—2	—
	30	24	0	+++	0	+++	0	+++	1—3	±(+)	1—2	±
	45	4—6	2	+(+)	0—1	++(+)	0	+++	2—3	—	2	—
	45	24	0	+++	0	+++	0	+++	0—2	+	1—2	±
Neohydrin	7	24	2	+	0—1	++	0	+++	2	—	2	—
	15	4—6	3	—	1	±	0—1	++	3	—	2	—
	15	24	1	++	0—1	++(+)	0	+++	1—3	±	2	—
	30	24	1	++	0—1	++(+)	0	+++	0—2	±(+)	1—2	—
	45	24	1	++	0	+++	0	+++	0—2	±(+)	1—2	±
Sublimate	5	24	2	±	1	+	0—1	++(+)	2—3	—	2	—
	10	24	2	±	1	+	0—1	++(+)	1—3	—	2	—
	15	4—6	3	—	1—2	±	0—1	++	3	—	2	—
	15	24	1—2	+	0—1	++	0	+++	1—2	±	1—2	±

curials. Meralluride and esidron inhibited totally the precipitation of formazan and induced necrosis throughout the length of this portion. The main damage by mercurophylline was located in the lowest and that of neohydrin, mersalyl and sublimate in the higher parts of the straight portion. These mercurials seemed to affect the lowest part of the straight terminal portion in a lesser degree. Exceptionally, the action of mercaptomerin on the straight terminal portion was relatively slight, its main effect being focused on the middle part of the proximal convoluted tubule.

Thin Segment of Henle's Loop. — This segment showed no demonstrable succinic dehydrogenase activity even in the control kidneys. Its cellular structure was not discernibly altered by the mercurials used. When necrosis was present in the proximal convoluted tubule casts were often seen in the lumen of the thin segment.

Thick Ascending Segment of Henle's Loop. — The succinic dehydrogenase activity of this segment was in general slightly to moderately reduced, especially in its upper part. Mercurophylline, however, caused throughout the ascending segment a marked inhibition of activity which was nearly complete within 24 hours. Mercaptomerin also affected the activity of the whole thick segment but in a lesser degree. In this segment the hemalum-eosin sections revealed no noticeable alterations.

Distal Convoluted Tubule and the Initial Part of the Collecting Tubule. — In these tubules no marked inhibition of succinic dehydrogenase and no obvious pathological changes could be observed.

Summarizing, the main effects of the different mercurials tested in our experimental conditions were focused as follows:

Mercurophylline. — The lowest part of the straight terminal portion of the proximal convoluted tubule and the ascending thick limb of Henle's loop (Fig. 3—4).

Mercaptomerin. — The middle part of the proximal convoluted tubule (Fig. 5—6).

Mersalyl. — The higher parts of the straight terminal portion of the proximal convoluted tubule (Fig. 7—8).

Esidron. — The straight terminal portion of the proximal convoluted tubule (Fig. 9—10).

Meralluride. — The whole proximal convoluted tubule (Fig. 11—12).

Neohydrin. — The straight terminal portion of the proximal convoluted tubule except its lowest part (Fig. 13—14).

Sublimate. — The higher parts of the straight terminal portion of the proximal convoluted tubule (Fig. 15—16).

Meralluride, mercaptomerin, neohydrin and esidron seemed to act at a higher level of the proximal convoluted tubule than mersalyl, sublimate and mercurophylline. Correspondingly, the kidneys of rats which had received mercurials acting at the high level were more swollen and had a pale cortex.

Functioning Blood Vessels. — The thioflavin-S-method for functioning blood vessels showed that most of the glomerular tufts in kidneys of rats treated with mercurials exhibited thioflavin-S-fluorescence but in contrast to the control rats it varied in intensity giving a more patchy picture. This indicated that the blood supply of some of the glomeruli was insufficient. The different mercurials exhibited no noticeable differences in the thioflavin-S-fluorescence of the various vascular areas of the kidney.

Sulfhydryl Groups. — The sections treated with Bennett's red sulfhydryl reagent showed no definite differences as compared with the controls.

Mercuric-ions. — All the efforts to localize free mercuric-ions failed even when mercuric chloride was tried to demonstrate.

COMMENTS

Sections from all kidneys were also incubated with BT. They exhibited a similar staining picture than NT-preparations. The observations presented are, however, derived solely from NT-sections since they display consistently uniform staining patterns in contrast to the occasionally patchy BT-preparations. On the other hand, the use of BT showed more clearly the inhibited sites since BT does not reveal a weak activity demonstrable with NT.

Since sulfhydryl groups are readily blocked by mercurials, a diminution in sulfhydryls could be expected. This potential change could not be detected with the method used, neither with the other methods employed earlier by us (10, 22).

DISCUSSION

Considerable work has been done to determine the exact segment of the nephron at which mercurials exert their action. Reasonably parallel physiologic methods have yielded superficially conflicting results, and theoretic constructions based mainly on indirect evidence have been put forward in support of both proximal and distal mercurial action [cf. the review of Pitts and Sartorius (15), and the monograph of Vogl (24)]. Weston, Grossman and Leiter (27) have stressed that »experimental analysis of renal regulation of sodium and chloride excretion is complicated by the fact that the functional contributions of the proximal and the distal tubular segments to the urinous electrolyte pattern cannot be readily separated. If electrolyte transport in one of these tubular sites could be significantly depressed without affecting the other it might be possible to clarify this tubular division of labor . . . The mercurial diuretics which reversibly inhibit tubular reabsorption of electrolytes and water would be valuable tools for such a dissection of tubular function if it could be established that such inhibition is localized to a particular tubular segment. However, the precise renal locus of this action of mercurials has not been unequivocally demonstrated.»

In histologic studies on kidney, corrosive sublimate has been found to affect mainly the straight terminal portion of the proximal convoluted tubule (2, 11, 20, 21). Using microdissection technique, Edwards (2) was able to demonstrate that in rats the lowest part of the straight terminal portion was least susceptible for sublimate-necrosis. Regarding the action of sublimate the present observations concur with those of Edwards.

A search through literature revealed relatively few histologic studies on the renal effects of the organic mercurials used as diuretics. Johnstone (6, 7) observed that the administration of merbaphen and mersalyl to rabbits produced lesions similar to those obtained with sublimate. In our experiments the behaviour of mersalyl was also most like of that of sublimate.

It is assumed that therapeutic doses of mercurial diuretics act at the same sites but that their effect is limited to mainly functional changes (24). There is ample evidence to indicate that mercurial diuretics interfere with certain enzyme systems controll-

ing the transport and selective reabsorption of electrolytes from the tubular lumen through the cells to the capillary blood, possibly by inactivating critical sulfhydryl groups (24). Succinic dehydrogenase, a sulfhydryl-requiring enzyme, is apparently involved in the production of energy for these absorption processes. Handley and Lavik (5) have demonstrated by manometric methods that the administration of meralluride to rats results in a marked decrease of succinic dehydrogenase activity in kidney without detectable inhibition of the enzyme in the heart and liver. Since this enzyme can also be studied with reliable histochemical methods, we have chosen it as subject of investigation in an effort to localize the action of mercurial diuretics within the nephron of rat kidney. Using mercurophylline we have previously (9, 10) found that it inhibits the succinic dehydrogenase activity in the medullary portions of tubules which are more adequately identified in this paper. The later observations of Wachstein and Meisel (25), and Rennels and Ruskin (16) regarding the proximal inhibition pattern of meralluride were also confirmed by us (23). Furthermore, the other mercurials tested seem to have peculiar inhibition patterns of succinic dehydrogenase at different levels in the tubule of rat kidney. In our experimental conditions, a slight to moderate depression of succinic dehydrogenase activity preceded microscopically discernible changes in the tubular cells, and in a later stage the marked to complete inhibition was well correlated to cellular damage.

Since the mercurial diuretics used differed only in their organic components, it can be assumed that the organic carriers modify the action of mercury presumably by influencing its release and pathway of excretion.

The evident functional implications of our observations remain to be established.

SUMMARY

Mercurophylline, mercaptomerin, mersalyl, esidron, meralluride, neohydrin and mercuric chloride in single subcutaneous doses of 7 to 45 mgHg/kg depressed markedly the histochemically demonstrated succinic dehydrogenase activity of rat kidney. The main inhibition was focused at different levels of the proximal con-

voluted tubule. Meralluride inhibited the whole proximal convoluted tubule, mercaptomerin the middle part of it, esidron the straight terminal portion and neohydrin, mersalyl and sublimate also this portion except the lowest part of it which, on the other hand, was inhibited by mercurophylline. Mercurophylline caused also a pronounced inhibition in the thick ascending segment of Henle's loop. This segment was only slightly to moderately depressed by the other mercurials, as well as the distal convoluted tubule which was least influenced. A noticeable inhibition of succinic dehydrogenase preceded histological lesions at the corresponding sites. The mercurials in the doses used caused also a defect in the blood supply of some of the glomeruli as revealed by the thioflavin-S-method for functioning blood vessels. The seven mercurials showed no noticeable differences in this respect. The attempts to localize free mercuric-ions and changes in sulfhydryl groups were unsuccesful. The organic components of mercurial diuretics seem to modify the sites where mercury exerts its action in rat kidney.

Acknowledgements. — The writers' thanks are due to Dr. H. L. Daiell, M.D., Lakeside Laboratories Inc. Milwaukee, Wisconsin, for the kind supply of Mercuhydrin Sodium and Neohydrin, to Professor A. Vartiainen, M.D., Messrs. Medica, Helsinki, for the kind supply of Novurit. We are also indebted to Dr. M. Wachstein, M.D., who sent to us his preparations for comparison, and to Professor M. J. Mustakallio, M.A., M.D., for his valuable criticism.

REFERENCES

1. Bennett, H. S.: The Demonstration of Thiol Groups in Certain Tissues by Means of a New Colored Sulfhydryl Reagent. — Anat. Rec. 1951:*110:*231.
2. Edwards, J. G.: The Renal Tubule (Nephron) as Affected by Mercury. — Am. J. Path. 1942:*18:*1011.
3. Farber, E., Sternberg, W. H., and Dunlap, C. E.: Tetrazolium Stains for Diphosphopyridine Nucleotide (DPN) Diaphorase and Triphosphopyridine Nucleotide (TPN) Diaphorase. — Proc. Soc. Exp. Biol. & Med. 1954:*86:*534.
4. Hand, W. C., Edwards, B. B., and Caley, E. R.: Studies in the Pharmacology of Mercury III. Histochemical Demonstration and Differentation of Metallic Mercury, Mercurous Mercury and Mercuric Mercury. — J. Lab. Clin. Med. 1943:*28:*1835.

5. Handley, C. A., and Lavik, P. S.: Inhibition of the Kidney Succinic Dehydrogenase System by Mercurial Diuretics. — J. Pharm. & Exp. Ther. 1950:*100:*115.
6. Johnstone, B. I., and Keith, H. M.: Toxicity of Novasurol (Merbaphen), its Action on the Kidney of the Rabbit. — Arch. Int. Med. 1928:*42:*189.
7. Johnstone, B. I.: The Comparative Toxicity of Merbaphen and Salyrgan — J. Pharm. & Exp. Therap. 1931:*42:*107.
8. Mager, M., McNary, W. F., Jr., and Lionetti, F.: The Histochemical Detection of Zinc. — J. Histochem. & Cytochem. 1953:*1:*493.
9. Mustakallio, K. K., and Telkkä, A.: Histochemical Localization of the Mercurial Inhibition of Succinic Dehydrogenase in Rat Kidney. — Science 1953:*118:*320.
10. Mustakallio, K. K., and Telkkä, A.: Effects of Mercurial Diuretics in Rat Kidney, Histochemical Studies. — Ann. Med. Exp. & Biol. Fenn. 1954:*32:*123.
11. Oliver, J., MacDowell, M., and Tracy, A.: The Pathogenesis of Acute Renal Failure Associated with Traumatic and Toxic Injury. Renal Ischemia, Nephrotoxic Damage and the Ischemuric Episode. — J. Clin. Invest. 1951:*30:*1305.
12. Padykula, H. A.: The Localization of Succinic Dehydrogenase in Tissue Sections of the Rat. — Am. J. Anat. 1952:*91:*107.
13. Pearse, A. G. E.: Histochemistry. — Churchill, London 1953. p. 421.
14. Pearson, B., and Defendi, V.: Histochemical Demonstration of Succinic Dehydrogenase in Thin Tissue Sections by Means of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl Tetrazolium Chloride Under Aerobic Conditions. — J. Histochem. & Cytochem. 1954:*2:* 248.
15. Pitts, R. F., and Sartorius, O. W.: Mechanism of Action and Therapeutic Use of Diuretics. — Pharm. Rev. 1950:*2:*161.
16. Rennels, E. G., and Ruskin, A.: Histochemical Changes in Succinic Dehydrogenase Activity in Rat Kidney Following Administration of Mercurial Diuretics. — Proc. Soc. Exp. Biol. & Med. 1954: *85:*309.
17. Rutenburg, A. M., Wolman, M.,, and Seligman, A. M.: Comparative Distribution of Succinic Dehydrogenase in Six Mammals and Modification in the Histochemical Technic. — J. Histochem. & Cytochem. 1953:*1:*66.
18. Schlegel, J. U.: Demonstration of Blood Vessels and Lymphatics with a Fluorescent Dye in Ultraviolet Light. — Anat. Rec. 1949: *105:*433.
19. Seligman, A. M., and Rutenburg, A. M.: The Histochemical Demonstration of Succinic Dehydrogenase. — Science 1951:*113:*317.
20. Simonds, J. P., and Hepler, O. E.: Experimental Nephropathies I. A Method of Producing Controlled Selective Injury of Renal Units by Means of Chemical Agents. — Arch. Path. 1945:*39:*103.

21. Suzuki, T.: Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen. — Gustav Fischer, Jena 1912.
22. Telkkä, A., and Mustakallio, K. K.: Sulfhydryl Groups and Succinic Dehydrogenase in Rat Kidney after Administration of Mercurial Diuretics, Histochemical Studies. — Experientia 1954:*10:*216.
23. Telkkä, A., and Mustakallio, K. K.: Proximal or Distal Mercurial Inhibition of Succinic Dehydrogenase in the Kidney Tubules of Rat. — Science 1955, in press.
24. Vogl, A.: Diuretic Therapy. — Williams & Wilkins, Baltimore 1953.
25. Wachstein, M., and Meisel, E.: On the Histochemical Localization of the Mercurial Inhibition of Succinic Dehydrogenase in Rat Kidney. — Science 1954:*119:*100.
26. Wachstein, M., and Meisel, E.: Influence of Experimental Renal Damage on Histochemically Demonstrable Succinic Dehydrogenase Activity in the Rat. — Am. J. Path. 1954:*30:*147.
27. Weston, R. E., Grossman, J., and Leiter, L.: The Effect of Mercurial Diuretics in Renal Ammonia and Titratable Acidity Production in Acidotic Human Subjects with Reference to the Site of Diuretic Action. — J. Clin. Invest. 1951:*30:*1262.